Sulfide

OBJECTIVE

Sulfide is a natural component of marine sediments, and may occur in concentrations that are toxic to marine organisms in toxicity tests. It is important to accurately measure concentrations of sulfide to account for any toxicity that may be attributed to them in solid phase toxicity tests. Sulfide analysis at Marine Pollution Studies Laboratory is a combination of the methylene blue and iodimetric methods from Fonselius (1985) and Standard Methods (APHA 1998).

PROCEDURE

Sample Preparation

- Reagents and chemicals needed: Zinc acetate
- Reagent preparation: 10.44g zinc acetate dihydrate (a.g.) is dissolved in one liter oxygen-free distilled water containing 2g gelatin. Oxygen-free water is prepared by bubbling with nitrogen gas for approximately 30 minutes. The gelatin is dissolved in the oxygen-free water by boiling.
- Sample preservation: Place sample into a scintillation vial (or other suitable small container with screw cap) already containing zinc acetate preservative. Ratio of sample to preservative should be 50:1. Keep samples in the dark to avoid degradation.

Standard Preparation

Reagents and chemicals needed:
Iodine (0.025N)
Phenylarsine oxide (0.025N)
Sodium thiosulfate (0.025N)
Starch indicator, (2%)
Sodium sulfide a.g. ($Na_2S \cdot 9H_2O$)
Potassium Iodide (KI)
6N H_2SO_4

- De-aerate one liter of water at the appropriate salinity by bubbling it with nitrogen gas for approximately 30 minutes.
- While de-aerating the seawater check the normality of the iodine by titrating with phenylarsine oxide (PAO). Put 10 mL iodine in a small beaker and titrate with PAO from a burette. As the solution turns to a pale yellow add 2 drops starch solution and titrate until clear. Repeat the process twice and calculate the normality using the following equation:

$$N=(\text{mean titration volume}/10\ \text{mL})(0.025N)$$

- Check the normality of sodium thiosulfate. Dissolve 2g KI in 175 mL distilled water. Add 2 mL 6N H_2SO_4. Add 20 mL potassium bi-iodate (0.2031g in 250 mL volumetric flask – made fresh). Titrate with STS from red to yellow and add 10 drops of starch. Titrate to clear.

N of STS = (20/mean)(0.025N)

- If not already prepared, make sulfide superstock by weighing out 1.5 g Na_2S into one liter distilled water. Prepare in a one-liter plastic volumetric flask with a screw cap. This superstock can be saved for several months.
- Dilute superstock 10x by measuring 50 mL into a 500 mL glass volumetric flask and diluting with de-aerated distilled water. Flush flask with Nitrogen gas before and after preparation. **NOTE**: from now on it is important to flush all glassware used with nitrogen gas before and after preparations.
- Titrate secondary stock 3 times in 50- mL volumes to determine concentration in mg/l.

Add 5 mL 0.025 iodine to 125-mL Erlenmeyer flask. Add 1 drop concentrated HCl. Add 50 mL secondary stock solution. Titrated with 0.025 STS. When color reaches pale yellow add 5 drops of starch indicator and titrate until clear.

$$\text{mg/l } S^{2-} = \frac{[(A \cdot B) - (C \cdot D)] \cdot 16000}{100 \text{ mL}}$$

A = mL of iodine
B = N of iodine
C = mL NaTS
D = N of NaTS

- **NOTE**: titrant volumes must not have a greater that 5% difference. If difference is greater than 5%, secondary stock must be prepared again.
- Prepare standards using secondary stock and de-aerated seawater with zinc acetate added (0.5488g in 1L) in nitrogen flushed 100 mL glass volumetric flasks.
- Calculate the amount of secondary stock to add to standard vials by dividing the standard concentration by the secondary stock concentration.

CALIBRATION CURVE PREPARATION AND SAMPLE READING

Equipment needed: Spectrophotometer, disposable cuvettes with 1-cm path length, and computer with graphing program.

Reagents and chemicals needed: Ferric chloride (100g in 40 mL water)
Amine sulfuric acid stock solution (contains N,N-dimethyl-P-phenylenediamine 27%, sulfuric acid 50%)
1+1 H_2SO_4.

- Reagent preparation: Dilute 25 mL amine sulfuric acid stock solution with 975 mL 1+1 H_2SO_4. Allow to cool and store in a dark glass bottle. Always prepare under a fume hood. If the ferric chloride reagent is unavailable from a commercial supplier, it can be made by dissolving 100 g $FeCl_3 \cdot 6\ H_2O$ in 40 mL water.

Creating a calibration curve

- Transfer 10 mL of each standard to a scintillation vial already containing 200 µL of 1:1 ferric chloride/amine sulfuric acid reagent. If samples have been preserved, an appropriate amount of preservative should also be added to the standards.
- Screw vial caps on tightly, invert once to mix.
- After 15 minutes, add 200 µL ammonium phosphate dibasic (dissolve 39.618 mL in 40 mL distilled water, transfer contents to 100 mL volumetric flask and dilute, store in fridge). Cap and invert.
- After another 15 minutes, color will develop completely and standards can be read on a spectrophotometer.
- Transfer standards to cuvettes and read on spectrophotometer at 680 nm wavelength.
- The spectrophotometer can be zeroed with the 0 standard or the 0 reading can be subtracted from other standard readings.
- Prepare the calibration curve using a computer-graphing program. **Important**: Make sure the generated line has an acceptable R^2-value (>0.9) before adding reagents to samples.

Measuring Samples

Reagents needed: ferric chloride, amine sulfuric acid, and ammonium phosphate dibasic.

- Arrange cuvettes in rack according to the number of samples that need to be analyzed. Add 80 µL of 1:1 ferric chloride/amine sulfuric acid reagent to each cuvette. Pipette 4 mL of sample into each cuvette, cap, and invert to mix.
- After 15 minutes add 80 µL ammonium phosphate dibasic. Cap and invert to mix.
- Allow color to develop for another 15 minutes before reading on the spectrophotometer at 680 nm.
- Samples can sit for up to 2 hours before reading
- At 30-minute intervals, re-read the standards for accuracy and precision.

Conversion of Total Sulfides to H_2S

Total sulfide readings are calculated from the standard curve and converted to H_2S using pH and the appropriate equilibrium coefficient (Savenko 1977). The following equation is used to determine what proportion of the total sulfide is H_2S:

$$[H_2S] = [S^{2-}] \times 1-[1/(1+10^{(pK-pH)})]$$

Sulfide pK values:

	0 ‰	20‰	35‰
15°C	7.06	6.91	6.81
20°C	6.98	6.83	6.73
25°C	6.90	6.75	6.65

Example: Temp = 15°C. $1-[1/(1+10^{(6.8-8)})] = 0.059$

Total sulfide = 2 mg/L $0.059 \bullet 2$ mg/L = 0.119 mg/L H_2S

pH = 8

Other K values for different salinities and temperatures can be found in Savenko (1977) and Standard Methods (APHA 1998).

QUALITY CONTROL

Standards are read throughout the analyses at 30-minute intervals. Accuracy and precision are calculated from these readings. Acceptable accuracy and precision are 10%. Volumes of titrant used for secondary stock verification must not differ by greater than 5%. Standard curve R^2 must be greater than 0.9.

REFERENCES

APHA. 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Public Health Association, Washington D.C.

Fonselius, S.H. 1985. Determination of hydrogen sulfide. In: Methods of Seawater Analysis. K. Grasshoff, M. Ehrhardt, K. Kremling (Eds.) 2nd Edition. pp. 73-81.

Savenko, V.S. 1977. Marine Chemistry: the dissociation of hydrogen sulfide in seawater. Oceanology, 16:347-350.